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A RARE CASE OF SPONTANEOUS RUPTURE OF THE CERVIX IN A NON-DESCRIPT BUFFALO

S. Manokaran

ABSTRACT

A rare case of spontaneous rupture of the cervix immediately after calving in a pluriparous non-descript buffalo and its successful treatment have been recorded.

Keywords: rupture of cervix, prolapse, non-descript buffalo, purse string suture, catgut

INTRODUCTION

Dystocia cases should be handled early and promptly on an emergency basis to save both the dam and the fetus. Prolonged dystocia, rough and improper use of obstetrical operations or improper manipulation of fetus leads to rupture of or damage to the reproductive organs (Roberts, 1971). This report records a rare case of spontaneous rupture of the cervix in a non-descript buffalo and its successful surgical management.

CASE HISTORY AND CLINICAL EXAMINATION

A pluriparous non-descript buffalo that had calved three times was brought to the PREPARE Veterinary Hospital* with the history of prolapse. The animal had normally delivered a live female calf 6 h previously without any assistance. Examination of the prolapsed mass showed a partially ruptured cervix.

TREATMENTS AND DISCUSSION

The animal was given epidural anesthesia with 5 ml of 2% xylocaine. The perineal region and the hanging mass were washed with running tap water and with 0.1% potassium permanganate solution. Examination of the prolapsed mass revealed a lengthy and pedunculated cervix with a rupture at the centre. The cervix was retracted and exteriorized through the vulva. The ruptured portion of the cervix was bluish and necrosed. The remaining portion of the cervix was apparently healthy and pale pink in color. The ruptured cervix was surgically incised between second and third annular ring and removed. The intact healthy portion of the cervix was sutured with purse string suture using catgut No. 2. Ice packs were applied to control the hemorrhage in the sutured area. The animal was administered inj. DNS (3 liters, i/v), inj. ampilcillin-cloxacillin (4 gm, i/v), inj. chlorpheniramine maleate (200 mg, i/m), inj. meloxicam (200 mg, i/m), inj. oxytocin (50 IU, i/v) and inj. calcium borogluconate (250 ml, i/v). A shark liver oil and suphadimidine power paste

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was applied on the cervix. The fluid therapy, antibiotic, antihistamine and anti-inflammatory were continued for five days and the animal had uneventful recovery.

The rupture of the uterus, cervix and vagina usually occurs during prolonged dystocia with fetal emphysema, torsion of uterus, improper manipulation and traction of fetus, accident in fetotomy operations, protruding portion of bones after fetotomy or inexpert manipulation of the fetus by a layman (Roberts, 1971). In bovines, forced traction of the fetus in a normal presentation may result in rupture of cervix due to sharp bony prominence. In the present case, as the delivery had been unassisted, the rupture of cervix would have occurred due to the pressure exerted by extremities and bony prominences of the fetus on the cervix. The long and pedunculated cervix might be a predisposing factor for the rupture.

REFERENCES

Arthur, G.H., D.E. Noakes, H. Pearson and T.J. Parkinson. 1996. *Veterinary Reproduction and Obstetrics,* 7th ed. WB Saunders Company Ltd., London.

Roberts, J. Stephen. 1971. *Veterinary Obstetrics and Genital Diseases,* 2nd ed. CBS Publishers and Distributors, New Delhi, India.

RUPTURE OF THE UTERUS WITH DISLOCATION OF THE FETUS INTO THE PERITONEAL CAVITY IN A NON-DESCRIPT BUFFALO: A CASE REPORT

S. Manokaran

ABSTRACT

A case report on rupture of the uterus with displacement of a live fetus into the peritoneal cavity and its successful treatment in a non-descript buffalo have been recorded.

Keywords: rupture of uterus, fetus, peritoneal cavity, non-descript buffalo, laparotomy

INTRODUCTION

Spontaneous rupture of a bovine uterus, possibly followed by partial or total displacement of the fetus into the peritoneal cavity is an uncommonly recorded complication of late pregnancy (Arthur *et al.,* 1996). The present report records a case of uterine rupture and subsequent escape of a live fetus into the peritoneal cavity.

CASE HISTORY AND CLINICAL OBSERVATION

A full term pregnant non-descript buffalo on its second calving was brought to the PREPAERE Veterinary Hospital with the history of continuous straining and anorexia for the previous two days. The owner observed relaxation of sacro-ischiatic ligament 72 h before and mild mucus discharge for the previous 10 h. The clinical examination of the animal revealed temperature of 38.7°C, restlessness, frequent lying down and getting up, enlarged vulval lips and colostrum secretion from the udder. Per vaginal examination revealed a four-finger dilated cervix. It was possible to reach internal os of cervix, but neither the water bag nor the fetal extremities could be palpated. On rectal examination, the presence of a fetus in the peritoneal cavity could be detected.

TREATMENTS AND DISCUSSION

It was decided to perform laporatomy. Tranquilization was achieved with triflupromazine hydrochloride (Siquil, Sarabhai Zydus) at the dose rate of 10 mg/45 kg (i/m). The animal was restrained in right lateral recumbency and the left flank was prepared for aseptic surgery. Laporatomy was performed through oblique incision. The skin, subcutis, abdominal muscles and parietal peritoneum were incised to reach the peritoneal cavity. A live female fetus (32.8 kgs) was delivered from the peritoneal cavity after ligation of the cord. The placenta appeared healthy and was removed. The gravid uterine horn was found to be involuted

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to a considerable degree with a longitudinal tear on its ventral surface. The uterine tear was repaired by Cushings followed by Lembert suture using catgut No. 2. The peritoneal cavity was flushed with 5 litres of normal saline and 5 gm of strepopenicillin was sprinkled in to the cavity. The abdomen was closed in a routine manner. The animal was administered inj. DNS (4 litres, i/v), inj. streptopenicillin (5 gm, i/m), inj. chlorpheniramine maleate (300 mg, i/m), inj. oxytocin (50 IU, i/v), inj. meloxicam (150 mg, i/m) and inj. belamyl (10 ml, i/m). The fluids, antibiotic, antihistamine and anti-inflammatory were continued for 5 days and recovery was uneventful.

Uterine rupture was usually the result rather than the cause of dystocia, the predisposing causes being uterine torsion, fetal emphysema, chronic perimetritis and abnormal fetal size and also the abnormal fetal movement. It may occur spontaneously or possibly be associated with violence in advanced pregnancy (Roberts, 1971). In many cases, the fetus dies with its membranes becoming walled off as a sterile foreign body in the ventral portion of the abdominal cavity (without external symptoms) or adhesions may develop with abdominal organs (with external symptoms). In the present case, however, the predisposing cause(s) could not be ascertained. The fetus survived in the peritoneal cavity because the cord was not twisted and the placenta was healthy and in such cases survival of the fetus in the peritoneal cavity for periods up to 7 days have been reported (Arthur *et al.,* 1996).

REFERENCES

Arthur, G.H., D.E. Noakes, H. Pearson and T.J. Parkinson. 1996. *Veterinary Reproduction and Obstetrics,* 7th ed. WB Saunders Company Ltd., London.

Roberts, J.S. 1971. *Veterinary Obstetrics and Genital Diseases,* 2nd ed. CBS Publishers and Distributors, New Delhi, India.

HYDROALLANTOIS IN BUFFALO: A CASE REPORT

Sharad Kumar, Utsav Sharma, A.K. Pandey, Sanjay Agrawal, R.B. Kushwaha and A.K. Tripathi

ABSTRACT

A case of hydroallantois in buffalo and successful treatment is reported.

Keywords: hydroallantois, buffaloes, gestational disorder, pregnancy

INTRODUCTION

Hydroallantois is one of the gestational disorder in which sudden increase in allantoic fluid occurs in allantoic cavity due to foetal membrane pathology leading to bilateral enlargement of abdomen (Roberts, 1971). This is more common last phase of third trimester in dairy and beef cattle and less so in buffaloes and heifers (Srinivas and Sreenu, 2006). The present report describes a case of hydroallantois in a 10-year-old buffalo that was 7 months pregnant.

CASE HISTORY AND CLINICAL EXAMINTATION

A 10-year-old buffalo that was 7 months pregnant was presented to the Veterinary Referral Hospital and Polyclinic, SKUAST-J with the history of sudden enlargement of abdomen in previous 4-5 days (Figure 1) and being unable to sit on its own. The buffalo was dull and depressed, its eyes ball were sunken, and its muzzle was dry.

The physiological parameters pulse rate, respiratory rate and rectal temperature were 105 per minute; 30 per minute and 102.3ºF, respectively. Ballottement of the abdomen failed any evidence of foetus. Extensive venous collateral circulation was noticed in the ventrolateral part of the abdomen. Rectal examination revealed highly distended uterus filling most of the pelvic cavity. Vaginal examination revealed completely dilated cervix and foetal membrane with fluid coming out in vagina. Based on history, symptoms and observations, the case was diagnosed as hydroallantois.

TREATMENTS AND DUSCUSSION

For removal of allantoic fluid, hand was inserted in vagina and protruded part of allantoic sac was broken out and keeping the hand in vagina, about 60 litres of allantoic fluid was slowly drained. After that separated part of placenta was removed from vagina manually (Figure 2) and again 80 litres of allantoic fluid was drained out from uterus slowly. The drained allantoic fluid was watery and amber in colour. After complete removal of allantoic fluid, foetus was palpated and delivered by rope traction tied in hind limbs (Figure 3). The remaining necrosed portion of placenta was removed manually. The animal was administered 10 litres of

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each 5% dextrose normal saline and normal saline solution intravenously. Injection of oxytetracycline 1500 mg (Terramycin, Pfizer) and injection of dexamethasone 60 mg (Dexasone, Zydus Animal Health Limited) were given intravenously whereas injection of Meloxicam 150 mg (Melonex, Intas Neovet) was administered intramuscularly. Bolus of Furazolidone and urea (Furea, Pfizer) was placed in uterus. The same treatment was continued for next 3 days except inj Dexamethasone. The animal recovered uneventfully.

In hydroallantois, accumulation of allantoic fluid is rapid due to placental abnormalities and possible interference with sodium metabolism at the cell level (Jackson, 1980). Hydroallantois is seen mostly in 8-9 months of pregnancy (Roberts, 1972); however, in the present case it was seen in 7 months of pregnancy and could be due to necrosed and oedematous placenta. Similar findings and cause also be earlier said by different researchers (Arthur, 1957, Roberts, 1972; Vandeplassche *et al.,* 1965). Sudden increase in fluid imposed pressure over diagphragm resulting in respiratory distress. The shifting of fluid from interstitial tissue or cell to cavity might have been responsible for dehydration, sunken eye, dullness and depression (Arthur *et al.,* 1989). Due to heavy bilateral distension of abdomen, ballotment was failed to revealed

Figure 1. A 10-year-old buffalo with hydroallantois. Note the distension of abdomen.

Figure 2. Heavy, necrosed and edematous foetal membrane after removal.

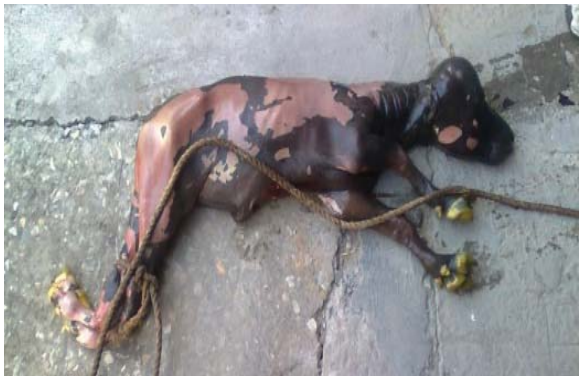

Figure 3. Dead foetus after removal. Note the non-hairy skin.

presence of foetus. Drainage of allantoic fulid or cesearean is the only treatment option (Arthur *et al.,* 1989). In present cases, foetus was removed after drainage of allantoic fluid per vaginum as the cervix was dilated at the time of examination and placenta and foetus was within the reach of hand. Inj. Dexona was administered to prevent the shock due to rapid drainage of fluid. Remaining treatment was given symptomatically. The present paper described and discussed a case of hydroallantois and its successful management in cattle.

REFERENCES

Arthur, G.H., D.E. Noakes and H. Pearson. 1989. *Veterinary Reproduction and Obstetrics*, ELBS, 118-120.

Arthur, G.H. 1957. Some notes on the quantities of fetal fluids in ruminants with special reference to Hydrops Amnii. *Brit. Vet. J*., **113**: 17.

Jackson, P.G.G. 1980. *Handbook of Veterinary Obstretics*. W.B. Saunders Company Limited, London. 221p.

Roberts, J.S. 1971. *Veterinary Obstetrics and Genital Diseases (Theiogenology)*, CBS Publishers and Distributors. 776p.

Srinivas, M. and Makkena Sreenu. 2006. Hydroallantois with foetal ascites in a buffalo. *Indian Vet. J.,* **83**(12): 1342-1343.

Vandeplassche, M., W. Oyaert, R. Bouters, C. Vandenhende, J. Spincemsille and J. Hermam. 1965. Uber die Eihautwassersucht beim Rind, Wiener Tierarztl. *Monatsschr.*, **5**(52): 461.

ATHELIA IN A GRADED MURRAH SHE BUFFALO: A CASE REPORT

R.V. Suresh Kumar, P. Sankar, P. Veena and N. Dhana Lakshmi

INTRODUCTION

Congenital abnormalities of the mammary system comprise absence of teats, glands, supernumerary teats and imperforate teats. Absences of teat is extremely rare, but isolated cases in which the teats were only represented by slight eminences have been met with (O' Connor, 1980). Verma *et al*. (1983) reported athelia in a Sahiwal heifer, and Baskal *et al*. (1979) also reported congenital absences of teats in a she goat. The present paper communicates a case of athelia in a cow.

CASE HISTORY AND CLINICAL OBSERVATIONS

A four-year-old graded Murrah she buffalo was presented to the Department of Veterinary Surgery and Radiology, College of Veterinary, Science, Tirupati with the history of absence of hind teats (Figure 1). Clinical examination of the udder by palpation showed normal udder tissue but the absence of two teats. Surgical correction was not possible.

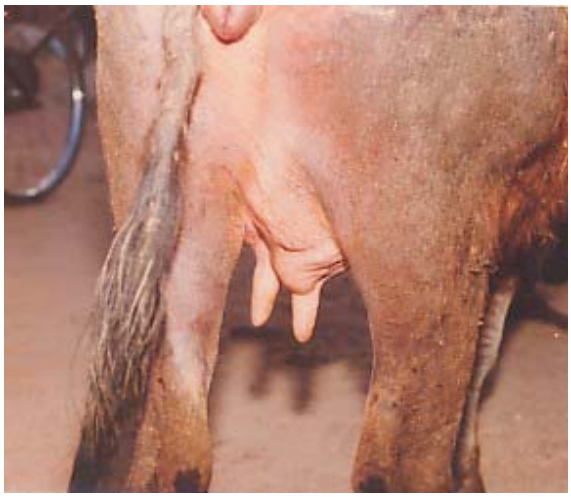

Figure1. Absence of hind teats.

REFERENCES

Baskal, D.K., A. Chakrabarti, R.N. Shom and A.K. Banerjee. 1979. *Indian Vet. J*., **56**(12): 1061.

O' Connor, J.J. 1980. *Text Book of Dollars Veterinary Surgery*, 4th ed. CBS, Publishers. 1,036p.

Verma, G.S., S. Tomar and O.S. Tomer. 1983. *Indian Vet. J*., **60**(7): 581.

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BACTERIAL ISOLATION AND ANTIBIOTIC SENSITIVITY TEST FROM URINE OF BUFFALO CALVES *(Bubalus bubalis)* AFFECTED WITH URETHRAL OBSTRUCTION

R.B. Kushwaha[1], Amarpal[1], H.P. Aithal[1], P. Kinjavdekar[2] and R. Rathore[3]

ABSTRACT

The study was conducted in 31 buffalo calves suffering from obstructive urolithiasis to isolate the bacteria and antibiotic sensitivity tests from their urine. Urine of the buffalo calves were collected via Foley's catheter or an infant feeding tube in a sterile test tube. Urine samples were subjected to culture isolation and antibiotic sensitivity tests. Out of 31 samples, 13 samples (41.94%) showed no growth up to 3 days of incubation period whereas the remaining 18 (58.06%) urine samples showed bacterial growth. The most commonly isolated organisms were *Escherichia coli, Staphylococcus species* and *Proteus species*. The cephalosporin and Fluoroquinolone groups of antibiotic were more sensitive against isolated organisms.

Keywords: antibiotic sensitivity test, buffalo calves, culture isolation, obstructive urolithiasis, urine

INTRODUCTION

Urethral obstruction is a fatal disease of cattle and buffaloes (Amarpal *et al.*, 2004). The disease may occur with or without urinary tract infection (Osborne *et al.*, 1981; Sharma *et al.*, 2006). Besides routine urine analysis, bacteriological studies of urine of the affected animal give an idea about nature of disease and help in planning an effective antibiotic therapy. The present paper describes the bacterial culture isolation and antibiotic sensitivity test from urine samples of buffalo calves *(Bubalus bubalis)* affected with urethral obstruction.

MATERIALS AND METHODS

Urine samples were collected from 31 buffalo calves affected with urethral obstruction in a sterilized test tube via either Foley's catheter while doing tube cystostomy or an infant feeding tube during urethrotomy or urethrostomy procedure. The collected urine samples were subjected to bacterial culture isolation and antibiotic sensitivity tests by standard procedure (Carter *et al.*, 1995; Bauer *et al.*, 1996).

RESULTS AND DISCUSSION

The different bacterial species isolated and antibiotic sensitivity test done against isolated

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[3]Indian Veterinary Research Institute (IVRI), Centre for Animal Disease Research and Diagnosis (CADRAD), Izatnagar, UP, India

bacteria have been depicted in Table 1. Out of 31 urine samples, 13 samples (41.94%) showed no growth up to 72 hours of incubation period whereas the remaining 18 (58.06%) samples showed bacterial growth. In the 18 positive samples, the different species of bacteria isolated were *Escherichia coli* (5; 16.12%), *Staphylococcus species* (4; 12.90%), *Proteus species* and *Pseudomonas aeruginosa* (each 3; 9.68%), and *Klebsiella species, Alkaligenes faecalis* and nonspecific gram positive (each 1; 3.23%).

The results showed that formation of urolith may occur with or without urinary tract infection. However, formation is more likely in case of urinary tract infection. Earlier reports said that struvite uroliths may form in sterile urine that contains high concentrations of magnesium, phosphorus and ammonium ions and is of neutral to alkaline pH but the probability of uroliths formation increased in presence of urinary tract infection particularly with urease producing bacteria viz. *Staphylococcus species* and *Proteus species* (Osborne *et al.,* 1981; Monoghan and Boy, 1990). The urease enzyme secreted by bacteria triggers the production of ammonia from urea, and hydrolysis of ammonia results in the production of the ammonium ion. Thus, the pH of urine in urinary tract infection further elicits production of more ammonium ion (Osborne *et al.,* 1981) Sharma *et al*. (2006) have also isolated *Escherichia coli* (57.10%), *Staphylococcus species* (28.5%) and *Klebsiella species* (14.4%) bacteria in cases of uroperitoneum and concluded that bacterial infection might be the favouring cause for high struvite uroliths that obstruct the urethra in buffalo calves.

The antibiotic sensitivity test against isolated bacteria varied greatly and did not show any specific trend. Similarly, great variation in the

Table 1. Bacteria isolated from urine samples and antibiotic sensitivity tests against isolated bacteria.

Sl No.	Organism isolated	No. of isolates	Sensitive Antibiotics
1	*Escherichia coli**	5 (16.12%)	Cefotaxime>Levofloxacin>Ciprofloxacin>ceftriaxone> Piperacillin>Nitrofurantoin
2	*Klebsiella* spp.	1 (3.23%)	Norfloxacin>ciprofloxacin
3	*Proteus* spp.*	3 (9.68%)	Ciprofloxacin>Gatifloxacin>Gentamicin
4	*Staphylococcus* spp. (epidermis-3, citrus-1)	4 (12.90%)	Norfloxacin>Getamicin>chloramphenicol>ceftriaxone> ciprofloxacin>ofloxacin>Gatifloxacin>cefotaxime
5	Non-specific gram +ve	1 (3.23%)	Norfloxacin>ciprofloxacin>Ofloxacin>Netallin> Nitrofurantoin>Nalidixic acid
6	*Pseudomonas aeruginosa*	3 (9.68%)	Cefotaxime>piperacillin>amikacin>ceftazidine> cefoparazone/sulbactum.
7	*Alkaligens faecalis*	1 (3.23%)	Cefotaxime>Netallin
8	No growth	13 (41.94%)	-

*One urine sample had mixed growth (*E coli* and *Proteus species*).

resistance to antibiotics was also observed. However, from the tested antibiotics, the cephalosporine and fluoroquinolone groups of antibiotics were more effective against the isolated bacteria, which corroborated the findings of Monoghan and Boy (1990) and Sharma *et al*. (2006).

From the present study it can be concluded that *Escherichia coli* and *Staphylococcus species* are the major bacteria that favour formation of more struvite uroliths in buffalo calves. The cephalosporine and fluoroquinolone groups of antibiotics were found more effective in treatment of the urolithiasis in the buffalo calves.

REFERENCES

Amarpal., P. Kinjavdekar, H.P. Aithal, A.M. Pawde, T. Singh and K. Pratap. 2004. Incidence of urolithiasis: A retrospective study of five years. *Indian J. Anim. Sci.*, **74**(2): 175-177.

Bauer, A., W. Kirby, J. Scherris and M. Tusk. 1996. Antibiotic susceptibility testing by a standardized single disc method. *Am. J. Clin. Pathol.*, **45**: 493-495.

Carter, G. R., M.M. Chengappa and A.W. Roberts. 1995. *Essentials of Veterinary Microbiology*, 5th ed. Williams and Wilkins, Philadelphia.

Monoghan, M.L. and M.G. Boy. 1990. Ruminants renal system: Diseases of the renal system, pp. 888-890. In Smith, B.P. (eds) *Large Animal Internal Medicine.* C.V. Mosby Company, Philadelphia, Toranto.

Osborne, C.A., J. S. Klausner, D.R. Krawiec and D.P. Griffith. 1981. Canine Struvite Urolithiasis: Problems and Their Dissolution. *J. Am. Vet. Med. Assoc.*, **179**(3): 239-244.

Sharma, P.D., K. Singh, J. Singh and A. Kumar. 2006. Bacteriological, biochemical and histopathological studies in uroperitoneum in buffalo calves *(Bubalus bubalis)*. *Indian J. Anim. Sci.*, **76**(2): 124-126.

EFFECT OF *Withania somnifera* AND *Azadiretica indica* AS ADJUNCT THERAPY ON HEMATO-BIOCHEMICAL PROFILES IN DIARRHEA BUFFALO CALVES

A.K. Tripathi[1] and V.S. Rajora[2]

ABSTRACT

Thirty (30) clinical cases of crossbred buffalo calves of either sex of 4-8 weeks of the age suffering from diarrhea were used for clinical validation of immunomodulation by *Withania somnifera* and *Azadiretica indica* at two different doses of 5 gm and 10 gm bid p.o. for 7 days. They were given along with standard treatment for diarrhea containing antimicrobials {trimethoprim and sulphamethoxazole} and rehydration therapy. Recovery was assessed on the basis of hematological parameters, protein profiles and total serum immunoglobulins. Significant increases in total leukocyte, percent lymphocyte and absolute lymphocyte were recorded in calves fed *Withania somnifera* (10 gm) and *Azadiretica indica* (5 and10 gm). Serum globulins were found significantly elevated in calves fed *Withania somnifera* (10 gm) and *Azadirectica indica* (10 gm). Albumin globulin ratio decreased in calves fed *Azadirectica indica* (10 gm). Total serum immunoglobulins were found significantly elevated in calves fed *Withania somnifera* (10 gm) and *Azadirectica indica* (10 gm). In the present study, it was found that powder of *Azadirectica indica* showed better results in comparison to *Withania somnifera* at the same dose regimen. However, the dose rate of 10 gm bid showed better results compared to 5 gm bid.

Keywords: Diarrhea, adjunct therapy, immunomodulation, Withania somnifera, Azadirectica indica

INTRODUCTION

Neonatal calf diarrhea is characterized by profuse watery diarrhea, progressive dehydration, acidosis and finally death within a few days (Aly *et al.,* 1993). The effectiveness of treatment and control of hard epidemics of diarrhoea in calves is frustrating and causes heavy economical losses (Radostits *et al.,* 1994). Common pathological lesions are dehydration, emaciation and a fluid filled intestinal tract with no other gross lesions (Brenner *et al.,* 1995). Neonatal calf diarrhea is one of the most common disease complexes and causes a great loss in vital body fluid and a decrease in the immune status of neonates which renders them more susceptible to different diseases and eventually results in their death (Hanif *et al.,* 1996). Treatment of diarrhea generally involves the alteration of diet, electrolyte and fluid replacement therapy and immunoglobulin therapy along with supportive therapy (Radostits *et al.,* 1994). Antimicrobial therapy may decrease bacterial pathogen numbers but its clinical efficacy is greatly impaired in the absence of adequate immune function. To improve the immune status of animals, a variety of

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synthetic immunomodulators have been tried as an adjunct therapy but the clinical efficacies of these immunomodulators are not up to the mark.

There are several herbs known to have immunomodulatory activity which can be used in diseases where immune system is depressed but their efficacy should be tried in clinical cases. The present study reports about a trial of two herbal immunomodulators, namely, *Withania somnifera* (roots) and *Azadiretica indica* (leaves), as an adjunct therapy in cases of diarrhea in buffalo calves.

MATERIALS AND METHODS

Thirty (30) clinical cases in crossbred buffalo calves of either sex of 4-8 week of the age suffering from diarrhea at the Instructional Dairy Farm (IDF) of G.B. Pant University of Agriculture and Technology, Pantnagar were used for clinical validation of immunomodulation by *Withania somnifera* and *Azadiretica indica* as an adjunct therapy. These calves, maintained under similar husbandry practices in separate cages and fed whole milk 10% of their body weight twice daily, were given standard treatment for diarrhea containing antimicrobials {trimethoprim and sulphamethoxazole}, and rehydration therapy. For the purpose of validation of immunomodulation by *Withania somnifera* and *Azadiretica indica* the calves were divided into the following five groups, each containing six calves.

Group 1: Untreated Control

Group 2: Powder of *Withania somnifera* 5 grams bid po daily for 7 days

Group 3: Powder of *Azadirectica indica* 5 grams bid po daily for 7 days

Group 4: Powder of *Withania somnifera* 10 grams bid po daily for 7 days

Group 5: Powder of *Azadirectica indica* 10 grams bid po daily for 7 days

The blood samples were collected before and on day 8 of institution of the therapy. The blood was aseptically collected from the jugular vein, using a 20 gauge needle for each calf, separately in 5 ml glass vials containing sodium ethylene diamine tetraacetate (EDTA) as an anticoagulant 1 mg /ml blood for hematological estimations. The blood for serum required for biochemical estimations was collected in a 10 ml capacity test tube with no anticoagulant and was allowed to stand undisturbed in slant position for about 3-4 h. The clot was retracted and the serum separated after rapid centrifugation. Extreme care was taken to prevent haemolysis. The serum thus collected was stored in a deep freeze at -200°C in glass vials, which were properly capped and labeled.

Hematological parameters were evaluated by method described by Jain (1986) within 2 h of collection of the samples. Protein profiles were estimated by the method described by Oser (1971). Total serum immunoglobulins were estimated by the zinc sulphate turbidity method (McEvan *et al*, 1969). Statistical analysis of all the data to test significance of means was done as per the method described by Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

Hematological profiles:

Perusal of Table 1 reveals that values of total leukocytes decreased in diarrheic calves in all the groups on day 8 post treatment compared to the values on day 0. Values of total leukocytes count were found to be significantly elevated after treatment with powder of *Withania somnifera* and

Azadiretica indica in calves of Groups 3, 4 and 5 as compared to Group 1 on day 8 post treatment. These findings of the present study suggest that powder of *Azadirectica indica* at dose rates of 5 and 10 gm and *Withania somnifera* only at a dose rate of 10 gm enhances total leukocyte count.

During present study, the decrease in percent neutrophils was observed in all the groups of calves on day 8 post treatment as compared to values on day 0. On day 8 the decrease in neutrophil count corresponded to increase in percent lymphocyte in all the treated groups. The findings of present investigation corroborates those of Donovan *et al*. (1998); however, Bukhari (2003) reported that after treatment, neutrophils first tend to increase but afterward become constant. The percent neutrophils was found to have decreased significantly in calves of various groups corresponding to increase in percent lymphocytes on day 8 post treatment.

In the present study, the percent lymphocytes increased in the diarrhea calves in all the groups whereas the absolute lymphocyte count decreased in Groups 1 and 2 and increased significantly in Group 5 on day 8 post treatment in comparison to their values on day 0. 0n day 8 post treatment, percent and absolute lymphocyte counts were found to have significantly increased in Groups 3, 4 and 5 in comparison to Group 1. A significant increase in percent and absolute lymphocyte counts in Groups 3, 4 and 5 in comparison to Group 2 and in Group 5 in comparison to Group 3 was recorded whereas increase in percent lymphocyte count and not in absolute lymphocyte count could be observed in calves of Group 5 in comparison to that of Group 4. These findings indicate that powder of *Azadirectica indica* stimulated the lymphocyte production both at 5 and 10 gm but *Withania somnifera* only at a dose rate of 10 gm bid.

The percent monocytes were decreased significantly on day 8 post treatment only in calves of Group 2 and 4 in comparison to that on day 0. A significant increase in Group 3 and decrease in Group 4 in comparison to Group 1 was recorded on day 8 post treatment. The percent eosinophil count declined significantly in Groups 1, 2 and 5 on day 8 post treatment in comparison to that on day 0. The variation observed in percent monocyte and eosinophil counts could not be explained for want of literature on this aspect.

Biochemical profiles

Perusal of Table 2 reveals that total serum proteins were significantly lower in all groups of diarrheic calves on day 8 post treatment compared to that on day 0. In the present study, significantly higher total serum proteins were recorded in diarrhea calves of Groups 3, 4 and 5 as compared to Group 1, and in Group 5 in comparison to Groups 2 and 3 on day 8 post treatment. These findings suggest that the administration of *Azadirectica indica* leaf powder at the dose of 5 gm (Group 3) and 10 gm (Group 5) and *Withania somnifera* root powder only at dose of 10 gm (Group 4) resulted in the increase in the total protein levels, and in terms of increase in total proteins, *Azadirectica indica* at the dose of 10 gm appeared superior. This increase in total proteins might have protected the animal from further disease and death. The findings of present investigation are in agreement with the observations made by Bukhari (2003) who reported that total serum proteins increases with the time and with the therapeutic agent.

Serum albumins levels were found to be reduced in all groups of diarrhea calves on day 8 post treatment compared to day 0. Serum albumin concentrations in diarrhea calves on day 8 post treatment significantly decreased in Group 5 and

Table 1. Hematological profiles in diarrheic buffalo calves.

Groups DPT	Total Leukocyte count (x10^9/l)		Differential Leukocyte Count (%)								Absolute Lymphocyte Count (x10^9/l)	
			Lymphocyte		Neutrophil		Monocyte		Eosinophil			
	0	8	0	8	0	8	0	8	0	8	0	8
1	12.35	9.39^{a}	53.50	64.41^{e}	45.08	34.43^{g}	0.52^{i}	0.37^{i,j,m}	0.77	0.44^{v,w,x,y}	6.61	6.05^{H}
	±0.27	±0.24	±0.37	±0.20	±0.41	±0.14	±0.11	±0.04	±0.15	±0.07	±0.17	±0.14
2	12.32	9.59^{a}	53.67	64.67^{e}	45.00	34.43^{g}	0.54	0.33^{j,n,o,p}	0.75	0.40^{v,z,A,B}	6.61	6.20^{H}
	±0.20	±0.24	±0.14	±0.23	±0.16	±0.31	±0.04	±0.06	±0.09	±0.05	±0.11	±0.14
3	12.40	10.34^{b,c}	53.79	65.49^{f}	44.71	33.22^{h}	0.56^{q}	0.43^{k,n,q,r,s}	0.79^{C}	0.58^{w,z,C,D,E}	6.67^{I}	6.79^{I,J}
	±0.25	±0.26	±0.28	±0.30	±0.19	±0.24	±0.04	±0.08	±0.08	±0.07	±0.13	±0.15
4	12.19	10.47^{b,d}	54.22	65.87^{f}	44.48	33.06^{h}	0.52	0.33^{l,o,r,t}	0.78^{F}	0.57^{x,A,D,F,G}	6.61^{K}	6.90^{J,K,L}
	±0.21	±0.29	±0.19	±0.22	±0.18	±0.27	±0.02	±0.03	±0.05	±0.05	±0.13	±0.19
5	12.12	10.82^{c,d}	53.99	67.24	44.53	31.46	0.56^{u}	0.43^{m,p,s,t,u}	0.80	0.58^{y,B,E,G}	6.54	7.27^{L}
	±0.17	±0.20	±0.19	±0.29	±0.11	±0.25	±0.04	±0.04	±0.08	±0.04	±0.11	±0.14

Means bearing common superscripts (a to L) did not differ significantly ($p<0.05$).

Group 1 : Untreated control

Group 2 : Calves treated with root powder of Withania somnifera 5 g bid po daily for 7 days.

Group 3 : Calves treated with leaf powder of Azadirectica indica 5 g bid po daily for 7 days.

Group 4 : Calves treated with root powder of Withania somnifera 10 g bid po daily for 7 days.

Group 5 : Calves treated with leaf powder of Azadirectica indica 10 g bid po daily for 7 days.

Table 2. Biochemical profiles in diarrheic buffalo calves of various groups.

Groups DPT	Total Serum Protein (g/l)		Serum Albumin (g/l)		Serum Globulins (g/l)		Albumin Globulin Ratio		Total Serum immunoglobulin (g/l)	
	0	8	0	8	0	8	0	8	0	8
1	75.22	65.70^{a}	43.33	38.30^{f,g}	31.89	27.23^{j,k}	1.36^{n}	1.40^{n,op}	15.87	17.36^{t,u}
	±0.55	±0.46	±0.64	±0.43	±0.80	±0.85	±0.05	±0.04	±0.20	±0.59
2	74.79	66.88^{a,b,c}	43.19	39.65^{h}	31.60	27.24^{j}	1.36^{q}	1.46^{o,q}	15.83	17.53^{t,v}
	±0.56	±0.81	±0.70	±0.54	±0.86	±0.85	±0.05	±0.06	±0.18	±0.56
3	75.28	68.22^{b,d,e}	41.53	39.08^{f,h}	33.40	29.14^{k,l}	1.24^{r}	1.35^{p,r}	15.64	18.33^{u,v}
	±0.34	±0.93	±0.33	±0.47	±0.37	±1.13	±0.01	±0.06	±0.10	±0.67
4	75.89	67.48^{c,d}	41.73	37.21^{g,i}	34.23	30.28^{l}	1.22^{s}	1.23^{s}	15.69	20.10
	±0.26	±0.52	±0.35	±0.19	±0.20	±0.52	±0.02	±0.02	±0.19	± 0.38
5	75.97	69.68^{e}	41.52	36.13^{i}	34.45^{m}	33.40^{m}	1.22	1.08	15.94	21.39
	±0.52	±0.37	±0.33	±0.17	±0.44	±0.23	±0.02	±0.01	±0.15	± 0.27

Means bearing common superscripts (a to v) did not differ significantly ($p<0.05$).

Group 1 : Untreated control

Group 2 : Calves treated with root powder of Withania somnifera 5 g bid po daily for 7 days.

Group 3 : Calves treated with leaf powder of Azadirectica indica 5 g bid po daily for 7 days.

Group 4 : Calves treated with root powder of Withania somnifera 10 g bid po daily for 7 days.

Group 5 : Calves treated with leaf powder of Azadirectica indica 10 g bid po daily for 7 days.

increased in Group 2 in comparison to Group 1. Low serum albumin levels were recorded in Groups 4 and 5 in comparison with Groups 2 and 3.

Serum globulin concentrations decreased significantly in diarrhea calves of Groups 2, 3 and 4 on day 8 post treatment compared to day 0. These findings of the present investigation are partly in agreement with the findings of Adam (1998) and Bukhari (2003) who reported that serum globulins decreased non significantly within time periods but remained constant within therapeutic agent.

The serum globulins were found significantly elevated in Groups 4 and 5 as compared with Group 1, in Groups 3, 4 and 5 in comparison with Group 2, and in Group 5 in comparison with Groups 3 and 4 on day 8 post treatments. These observations indicated that administration of *Azadirectica indica* at the dose of 5 gm as well as 10 gm and *Withania somnifera* at the rate of 10 gm bid increased the serum globulins significantly.

The albumin globulin ratio decreased significantly in Group 5 on day 8 post treatment in comparison to the values on day 0. This suggested that only *Azadirectica indica* powder at the dose of 10 gm could decrease the A: G ratio, possibly due to the greater increase in serum globulins. Perusal of Table 2 reveals that on day 8 post treatment, the albumin globulin ratio decreased significantly in Groups 3, 4 and 5 in comparison with Group 2, in Groups 4 and 5 in comparison with Group 3 and in Group 5 in comparison with Group 4. These observations suggest that the treatments given Groups 5, 4, 3 and 2 were in order of merit in terms of serum globulin concentrations.

In the present study, total serum immunoglobulins were found to have significantly increased in all the groups on day 8 post treatment as compared to the values on day 0. This increase in total serum immunoglobulins might possibly be due to the body's immune response against either the infectious agent or the powder administered in the present study. Boyd *et al*. (1974) found that those calves which have high total serum immunoglobulins did not suffer with diarrhoea. Significantly increased total serum immunoglobulins on day 8 post treatment were recorded in Groups 4 and 5 when compared with diarrhoea calves of Groups 1, 2 and 3 and the levels in Group 5 were highest than those in Group 4. These findings suggest that the highest serum immunoglobulins were recorded in calves of Group 5 administered *Azadirectica indica* at the dose of 10 gm followed by Groups 4, 3 and 2, fed *Withania somnifera* at rate of 10 gm, *Azadirectica indica* at the dose of 5 gm and *Withania somnifera* at the dose of 5 gm, respectively. Boyd *et al*. (1974) found that those calve which had high total serum immunoglobulins were not affected with diarrhoea. The feeding of *Withania somnifera* and *Azadiretica indica* might have increased the serum immunoglobulin, and therefore, the two herbal preparations can be used as immunomodulator to protect the animal from ill effect of diarrhea.

In the present study, powder of *Azadirectica indica* showed better results in comparison to *Withania somnifera* at the same dose regimen. However, the dose rate of 10 gm *bid* showed better results compared to 5 gm *bid*.

REFERENCES

Aly, A.O., Z.H. Abd-el-Wahed, K. Kohilo and A.R. El-Sheikh. 1993. Some studies on clinical hematological and biochemical changes diarrheic buffalo calves with reference to hygienic conditions. *Assiut Vet. Med. J*., **35**: 91-104.

Adam, S.E.T. 1998. Toxicity of Rhazzya stricta to sheep. *Vet. Hum Toxicol*., **40**: 68-69.

Boyd, J.W., J.R. Baker and A. Leyland. 1974. Neonatal diarrhoea in calves. *Vet. Rec*., **95**: 310-313.

Brenner, J., D. Elad, H.M. Van, A. Markovic, S. Perl and M. Van Ham, 1995. Microbiological findings in young calves suffering from neonatal diseases. *Israel J. Vet. Med*., **50**: 21-24.

Bukhari, S.M.H. 2003. Effect of chemoprophylaction against neonatal buffalo calf diarrhoea over normal physiology of buffalo neonates. *Online Journal of Biological Sciences,* **3**(3): 282-286.

Donovan, G.A., I.R. Dohoo, D.M. Montgommery and F.L. Bennett. 1998. Association between passive immunity and morbidity and mortality in diary heifers in Florida, USA. *Prev. Vet. Med.*, **34**: 34-46.

Hanif, M.K., M.A. Khalil, M. Rafiq, M. Saleem and M. Anees. 1996. *Goat Farming*, pp: 14.

Jain, N.C. 1986. *Schalm's Veterinary Haematology*, 4th ed. Lea and Febiger, Philadelphia. 41.

Oser, B.L. 1971. *Hawk's Physiologial Chemistry*, 14th ed. Tata McGraw Hill Publishing Co., New Delhi. 952-955.

McEwan, A.D., W. Fisher, I.F. Selman and W.J. Penhale. 1969. A turbidity test for the estimation of immune globulin levels in neonatal calf serum. *Clin. Chim. Acta*., **27**: 155-163.

Radostits, O.M., D.C. Blood and C.C. Gay. 1994. *Veterinary Medicine*. Baillier Tindall. 703-707.

Snedecor, G.W. and W.G. Cochran. 1994. *Statistical Methods*, 8th ed. Iowa State University Press. Ames. Iowa U.S.A.

EFFECT OF VITRIFICATION ON *IN VITRO* MATURED BUFFALO OOCYTES

Ashish Mishra*, Vikash Chandra and G. Taru Sharma

ABSTRACT

To find out the effect of vitrification on *in vitro* matured buffalo (*Bubalus bubalis*) oocytes, quality oocytes were collected from slaughterhouse ovaries and matured using the standard IVMFC protocol of our laboratory. Matured oocytes were vitrified by using 20% ethylene glycol + 20% dimethyl sulfoxide (DMSO) + 0.6M sucrose for a period of a minimum of 7 days in liquid nitrogen (LN2) at -196^{0}C. Post thaw recovery of oocytes was 81.35%, and 18.60% of oocytes were lost during recovery. Major cryo injuries noted for these oocytes were zona breakage, crack in zona, leakage of the cellular contents and cytoplasmic shrinkage.

Keywords: vitrification, *in vitro* oocytes, buffalo

INTRODUCTION

In vitro embryo production procedure has been successfully been used for routine production of embryos from slaughterhouse ovaries in buffaloes (Mishra *et al*., 2010) but poor recovery of total and acceptable quality oocytes severely hampers the practical application of these techniques in buffalo. Because of poor adoption of artificial insemination (AI) and low efficiency of multiple ovulation with embryo transfer (MOET), there is an increasing interest in large scale *in vitro* production of buffalo embryos (Madan *et al*., 1996). Capability in cryopreservation or vitrification of oocytes could increase their availability for a broad range of reproductive technology applications in the buffalo. Over the past decade, extensive research has been conducted to find out the most appropriate cryoprotectant additives to preserve the oocytes and embryos. Successful results through vitrification of oocytes has already been reported in many studies (Sharma *et al*., 2006; Mishra *et al*., 2009). The objective of this study was to find the impact of vitrification on *in vitro* matured buffalo oocytes in terms of morphological changes.

MATERIALS AND METHODS

Oocyte collection and *in vitro* maturation (IVM)

Buffalo ovaries after collection from a local slaughterhouse were transported to the laboratory in 0.9% normal saline fortified with gentamycin (50 µg/ml) within 3 h of slaughter at 37°C in a thermos flask. Oocytes were aspirated from follicles (2-8 mm in diameter) with an 18 gauge needle attached to a 5 ml syringe with oocyte collection media containing 3 mg of bovine serum albumin (BSA) per ml of Dulbecco's phosphate buffer saline (DPBS). Aspirated oocytes were

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classified according to their number of cumulus layers and homogenicity of cytoplasm under a zoom stereomicroscope. Quality oocytes were matured in tissue culture medium-199 (TCM-199) with 10% fetal calf serum (FCS) and BSA (3mg/ml) at 5% CO_2, 15% O_2, 38.5°C for 24 h. To check the nuclear maturation, a few oocytes were taken out and stained with aceto-orecein staining to evaluate the metaphase-II (MII) stage, as described by Sharma *et al*., 1996.

Vitrification of *in vitro* matured oocytes

Two vitrification solutions (VS) were prepared in media consisting of TCM-Hepes with 20% FCS. Vitrification solution 1 (VS1) consisted of 10% ethylene glycol+ 10% dimethyl sulfoxide (DMSO) and vitrification solution 2 (VS2) consisted of 20% ethylene glycol + 20% DMSO + 0.6 M sucrose. The matured oocytes with cumulus cells were exposed to VS1 for 1 minute followed by 25 seconds in VS2. The matured oocytes in VS2 were loaded in 0.25 ml French straw and plunged into liquid nitrogen (LN2) at -196°C. The straws were thawed after a storage period of 7 days by transferring them into a water bath at 37°C for 30 seconds; then, the cryoprotectants were removed by exposing the matured oocyes to sucrose (1 mol/l).

RESULTS AND DISCUSSION

After *in vitro* maturation, oocytes were vitrified and stored for a period of at least one week. After seven days or more, these oocytes were thawed and post thaw recovery was recorded (Table 1). Post thaw recovery was 81.35%, and 18.60% oocytes were lost during recovery. Major cryo injuries noted for these oocytes were: zona breakage, crack in zona, leakage of the cellular contents and cytoplasmic shrinkage. Overall damaged oocytes were recorded as 25.71%, and the percentage of the normal oocytes was found to be 74.28%. That there was no significant difference between the recovery percentage and normal oocyte percentage post vitrification supports few previous finding (Nowshari *et al*., 1994; Lognathasamy, 2004). Loss of oocytes during the process of freezing and thawing is well documented in several studies. Such loss could be due to sticking of oocytes to the inner wall of straws or to disintegration of oocytes as an impact of vitrification. Similar types of damage due to vitrification were also observed in earlier

Table 1. Impact of Vitrification on in vitro matured buffalo oocytes.

Experiment	Total vitrified oocytes	Recovered oocytes	Oocytes lost	Normal Oocytes	Damaged Oocytes
1	40	29	11	18	11
2	45	35	10	29	6
3	50	43	7	27	16
4	35	27	8	17	10
5	45	41	4	39	2
Total	215	175 (81.35%) 35.00±3.16a	40 (18.60%) 8.00±1.22	130 (74.28%) 26.00±4.02a	45 (25.71%) 9.00±2.37

Significance observed at $p<0$.

studies in buffalo (Loganathasamy 2004; Dhali *et al*., 2000) and mouse oocytes (Sathananthan *et al*., 1988).

REFERENCES

Dhali, A., R.S. Manik, S.K. Das, S.K. Singla and P. Palta. 2000. Post-vitrification survival and *in vitro* maturation rate of buffalo (*Bubalus bubalis*) oocytes effect of ethylene glycol concentration and exposure time. *Anim. Reprod. Sci.*, **63**: 159-165.

Lim, J.M., Y. Fukui and H. Ono. 1992. Developmental compentence of bovine oocytes frozen at various maturation stages followed by in vitro maturation and fertilization. *Theriogenology*, **37**: 351-361.

Loganathasamy, K. 2004. Developmental competence of buffalo oocytes vitrified of different stages of maturation *in vitro*. Ph.D. Thesis submitted to IVRI, Izatnagar.

Madan, M.L., S.K. Das and P. Patta. 1996. Application of reproductive technology to buffaloes. *Anim. Reprod. Sci.*, **42**: 299-306.

Mishra, A., V. Chandra and G.T. Sharma. 2010. Effect of epidermal growth factor on *in vitro* maturation of buffalo oocytes and subsequent development with insulin-like growth factor-1 and β-mercaptoethanol. *Indian J. Anim. Sci.*, **80**(8): 17-20.

Mishra, A., G. T. Sharma and G. S. Kumar. 2009. Expression of Connexin 43 (Cx43) and Poly A Polymerase (PAP) genes in vitrified *in vitro* matured buffalo oocytes. *Indian J. Anim. Physiol.*, **4**(1): 6-10.

Nowshari, M.A., P.L. Nayaudu and J.K. Hodges. 1994. Effect of cryoprotectant concentration, equillibriation time and thawing procedure on survival and development of rapid frozen-thawed mouse oocytes. *Theriogenology*, **42**: 1193-1204.

Sathananthan, A.H., S.C. Nag, A.O. Throunson, A., Bongso, S.S. Ratnam, J. Ho, H. Mok and M.N. Lee. 1988. The effects of ultrarapid freezing on meiotic and mitotic spindles of mouse oocytes and embryos. *Gamete Res.*, **21**: 385-401.

Sharma, G. T., S. D. Kharche and A. C. Majumdar. 2006. Vitrification of *in vitro* matured goat oocytes and the effect of *in vitro* fertilization. *Small Ruminant Res.*, **64**: 82-86

Sharma, G.T., A. C. Majumdar and S. W. Bonde 1996. Chronology of maturational events in goat oocytes cultured *in vitro*. *Small Ruminant Res.*, **22**: 25-30.

SERUM BIOCHEMICAL ALTERATIONS IN NATURALLY ACQUIRED BUBALIAN TROPICAL FASCIOLOSIS

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ABSTRACT

Buffaloes are the important multipurpose farm animals in the Indian sub-continent, contributing significantly to meat and milk production. Tropical fasciolosis caused by *Fasciola gigantica* is regarded as one of the most important diseases of buffaloes in humid tropical regions of the world. Bio-chemical changes are regarded as important indicators for actual pathogenesis and clinical diagnosis. Serum samples were collected randomly from buffaloes (n=100) slaughtered at a local abattoir. Serum biochemical parameters have been compared between the *Fasciola* positive and the negative groups. The present study revealed that there was a highly significant difference ($P<0.01$) between the bilirubin direct and bilirubin total levels in the positive and negative groups. The albumin (3.44±0.09 g/dl) and total protein (12.09±0.41 g/dl) in affected animals were reduced when compared with the negative group. Aspartate aminotransferase was reported higher in positive animals (233.11±6.35 IU/L) as compared to negative (217.09±6.32 IU/L). Alkaline phosphatase values were found similar in both the groups. The findings of this study revealed that detection of biochemical alterations especially bilirubin can be used as an early indicator of pathophysiological changes caused by *F. gigantica* in buffaloes.

Keywords: biochemical changes, buffalo, fasciola gigantica, aspartate aminotransferase, alkaline phosphatase

INTRODUCTION

Tropical fasciolosis in buffaloes is asymptomatic, subclinical and/or chronic form of the disease, adversely affecting their reproductive cycle, weight gain, food conversion efficiency and productivity. The host suffers from unnoticed ill effects of the disease for a prolonged period before the disease is detected at a veterinary clinic and/or the abattoir (Edith *et al*., 2010). Traditionally, *Fasciola gigantica* infection is diagnosed by faecal examination. This has several disadvantages such as time consumption, requirement of large volume of faeces, inability to detect prepatent and ectopic infections and high false negative percentage in chronic infections due to intermittent shedding of eggs in faeces. Furthermore, clinical disease can

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occur as early as 3-4 weeks post infection while faecal examination can confirm the diagnosis only after 13 weeks. Serological tests are highly sensitive epidemiological tools for the detection of the disease, but their application is limited by cost and expertise in most developing countries. Serum biochemistry of infected animals can be a good indication of the degree of damage to the host and the severity of infection (Otesile *et al*., 1991). Biochemical changes induced by *F. gigantica* in experimentally infected animals have been studied extensively. However, similar information on natural *F. gigantica* infection in buffaloes is scanty (Swarup and Pachauri, 1987; Chaudhri *et al*., 1988; Raval, 2006). The present investigation records the changes in serum biochemical constituents of buffaloes naturally infected with *F. gigantica*.

MATERIALS AND METHODS

Samples of 5 ml of blood was aseptically collected from the jugular vein without anticoagulant randomly from slaughtered buffaloes (n=100) in sterile tubes. The blood was allowed to clot at room temperature in an inclined position and the serum separated was transferred to Eppendrof's tubes. These tubes were transported in ice packed conditions to the working lab. The sera were then centrifuged at 2500 rpm for 10 minutes to remove suspended RBC's, and clear sera was labeled as Fasciola positive (referral) and negative (control) sera samples prior to their storage at -20°C in deep freeze. Animals whose livers were observed to be infected with *Fasciola* were considered as the positive group (n = 54) whereas the negative group (n= 46) consisted of the animals which did not have *Fasciola* infection at necropsy. Biochemical parameters were analyzed by using diagnostic kits in an auto analyzer and compared by unpaired t-test (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

The mean values of the *Fasciola* positive and the negative buffaloes are presented in Table 1.

Domestic ruminants experimentally infected with *F. gigantica* exibit substantial changes both in their serum proteins as well as serum activities of hepatic enzymes. These biochemical changes take place in two stages. The first stage coincides with the period of fluke migration. Synchronously with the onset of traumatic hepatitis and progressive hypoalbuminaemia, the serum concentrations of aspartate aminotransferase (AST) progressively increase and attain the highest level by week 6 post infection. The second stage is associated with the presence of adult parasites in the bile ducts and is attended by further deterioration in albumin. The elevated AST declines significantly, and the alkaline phosphatase (ALP) show elevated trends from the 6th week post infection onwards (Mbuh and Mbwaye, 2005; Ahmed *et al*., 2006; Edith *et al*., 2010).

The biochemical parameters of the buffalo naturally infected with *F. gigantica* (irrespective of the stage) revealed a significant increase in bilirubin (direct and total) in the *Fasciola* positive buffaloes as compared to that of *Fasciola* negative buffaloes. These findings were in agreement with the observations of Raval (2006). Cholestasis develops during the migration of immature flukes through the liver and attachment of mature flukes in bile ducts as well and is related to alterations of the structural integrity of hepatocytes (Yang *et al*., 1998). Total protein and albumin levels in the

positive group were reduced as compared with the negative group. The decrease in these biochemical constituents is because of their reduced synthesis and increased plasma leakage into the gut. High AST value in *Fasciola* positive buffaloes may be due to extensive hepatic cell damage by the migrating flukes. This enzyme is specific for the liver and higher values indicate soft tissue damage. Increases in AST activities accompanied by reduction in bile flow and increase in bilirubin concentration were also found in sheep (Ferre *et al*., 1995). Contrary to earlier observations of marginal increases in ALP concentrations in *F. gigantica* affected buffaloes at necropsy (Swarup and Pachauri, 1987), no significant difference could be depicted in the ALP levels of the positive and negative groups. The reason for this may be that most of the cases included here were in an acute stage of the disease.

The findings of this study revealed that biochemical parameters, especially bilirubin, are early indicators of pathophysiological changes caused by *F. gigantica* in buffaloes. They may be useful for disease forecasting, assessment of the efficacy of drugs against early stages of the parasite and in time application of control strategies.

REFERENCES

Ahmed, M.I., A.G. Ambali and S.S. Baba. 2006. Haematological and biochemical responses of balami sheep to experimental *Fasciola gigantica* infection. *J. Food Agric. Environ.*, **4**(2): 71-74.

Chaudhari, S.S., V.M. Mandokhot, R.P. Gupta and C.L. Yadav. 1988. Haematological and biochemical observations in buffaloes naturally infected with *Fasciola gigantica*. *Indian Vet. J.*, **65**: 23-27.

Edith, R., R. Godara, R. L. Sharma and M. Thilagar. 2010. Serum enzyme and hematological profile of Fasciola gigantica immunized and experimentally infected riverine buffaloes.

Table 1. Mean values of biochemical parameters in Fasciola positive and negative buffaloes (Mean±SE).

S. No.	Parameters	Fasciola Negative	Fasciola Positive	t-stat
1	Albumin (g/dl)	3.55±0.11	3.44±0.09	0.79
2	Globulin (g/dl)	9.29±0.50	8.65±0.41	0.99
3	A : G	0.43±0.02	0.51±0.05	1.38
4	TSP (g/dl)	12.58±0.53	12.09±0.41	1.15
5	BID(mg/dl)	1.42±0.03	1.73±0.04	5.86**
6	BIT(mg/dl)	1.67±0.04	1.99±0.04	5.42**
7	AST (IU/L)	217.09±6.32	233.11±6.35	1.78
8	ALP(IU/L)	75.61±2.24	75.43±3.99	0.04

ALP-alkaline phosphatase, TSP-total serum protein, A G ratio-albumin globulin ratio, BID-bilirubin direct, BIT-bilirubin total, AST-aspartate aminotransferase.

** Significant at P<0.01

Parasitol. Res., **106**(4): 947-956.

Ferre, I., P. LoÂpez, M. Gonzalo-Orden, M.D. Julian, F.A. Rojo and J. GonzaÂlez-Gallego. 1995. The effects of subclinical fasciolosis on hepatic secretory function in sheep, *Parasitol. Res.*, **81**: 127-131.

Mbuh, J. and J. Mbwaye. 2005. Serological changes in goats experimentally infected with in buea sub-division of s.w.p. cameroon. *Vet. Parasitol.*, **131**(3-4): 255-259.

Otesile, E.B., B.O. Fagbemi and O. Adeyemo. 1991. The effect of T. brucei infection on serum biochemical parameters in boars on different planes of dietary energy. *Vet. Parasitol.*, **40**: 207-216.

Raval, S.K. 2006. Study on some biochemical changes in fasciolosis in rural buffaloes. *Vet. World,* **5**(2): 39-43.

Snedecor, G.W. and W.G. Cocharan. 1994. *Statistical Methods*, 7th ed. The Iowa State University Press, Ames, Iowa, USA.

Swarup, D. and S.P. Pachauri. 1987. Biochemical, histopathological and histochemical changes in liver and buffaloes affected by fasciolosis. *Indian J. Anim. Sci.*, **57**: 1077-1082.

Yang, Q., W.H. Mao, I. Ferre, J.E. Bayon, X.Z. Mao and J.G. Gallego. 1998. Plasma aspartate aminotransferase (AST), glutamate dehydrogenase (GLDH) and gamma-glutamyl transpeptidase (GGT) activities in water buffaloes with experimental subclinical fasciolosis. *Vet. Parasitol.*, **78**(2): 129-136.

POLYCLONAL ANTIBODY PRODUCTION AGAINST SOMATOTROPIN TO ESTIMATE SOMATOTROPIN LEVELS IN BUFFALOES BY ELISA

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ABSTRACT

The aim of the study was to produce sensitive and specific polyclonal antibodies against somatotropin (ST) to be used for standardization of an ELISA to measure growth hormone (GH) level in serum and milk of lactating buffaloes. Recombinant bovine somatotropin (rbST), kindly donated by NHPP, California, USA was injected both in chicken and rabbit emulsified with Freund's complete adjuvant followed by injection with Freund's incomplete adjuvant. Polyclonal antibodies in sera were detected by indirect ELISA and separated by the salt precipitation method. Immunoglobulins were purified by ion exchange chromatography on a DEAE Sepharose column followed by affinity chromatography. Purity of anti rbST polyclonal antibodies were determined by SDS-PAGE analysis and western blot. Antibody content of the sample was determined by Lowry's method.

Keywords: somatotropin, polyclonal antibody, serum, milk, buffaloes

INTRODUCTION

Growth hormone (GH), or somatotropin (ST), is a protein of 22 kDa synthesized and secreted from the anterior pituitary and has been popularized for use in many areas from basic science to commercial use. The effect of GH in lactating animals has been the subject of scientific interest for many years. After parturition, an increase in the demand for nutrients to provide a suitable substrate for milk production keeps the animal in stress that causes a metabolic imbalance and a sudden change in the hormonal status of the animal. The regulatory hormones which exert their influences on galactopoietic process in the epithelial cells of the mammary gland are insulin, prolactin, GH, thyroid hormone and glucocorticoids (Tucker, 1981). Out of these hormones, GH along with insulin plays a significant role in repartitioning of the major nutrients in the mammary gland for milk synthesis. GH is secreted in pulsatile fashion with a dominant periodicity of approximately 3 h. There are assays like enzyme-linked immunosorbent assay (ELISA) and radio immuno assay (RIA) available to measure GH levels in the sera of different species (Secchi *et al*., 1999; Prakash *et al*., 2003). To standardize an ELISA to measure GH level in the serum and the milk of lactating buffaloes, our target was to produce polyclonal antibodies against ST.

MATERIALS AND METHODS

Recombinant bovine somatotropin (rbST,

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Monsanto) was kindly donated by Dr. A. F. Parlow, National Hormone and Peptide Programme (NHPP), California, USA.

Preparation of anti-rbST IgG antibodies

Antibodies against rbST were raised both in chickens and rabbits. A total of six male chickens and three rabbits were used for this purpose. Both chickens and rabbits were injected with 400 µg of rbST (Monsanto) emulsified with Freund's complete adjuvant (FCA, Sigma Chemical) by the intramuscular route at multiple sites of the thigh followed by one booster of 100 µg rbST emulsified with Freund's incomplete adjuvant (FIA, Sigma Chemicals) at 2 week intervals. Finally two more boosters were given at 1 week intervals. The bleeding was performed 1 week after the last booster. The sera were collected by centrifuging the blood at 3500 rpm for 15 minute at 4°C.

Detection anti rbST antibodies

Anti rbST antibodies in the chickens and the rabbits were detected by indirect ELISA as described by Wilkin *et al*., 1989 with some modification.

Separation of immunoglobulins from sera

Chicken sera frequently contain high levels of lipids and lipo-proteins, which make subsequent purification difficult. To avoid interference by lipids and lipoproteins, delipidation of anti rbST hyper immune serum was performed using dextran sulphate and calcium chloride (Masseyeff *et al*.,1965). Chicken gamma globulins were separated by sodium sulphate fractionation as described by Lebaco (1979). Immunoglobulins from the rabbit sera were separated by the 45% ammonium sulphate precipitation method (Weir, 1996).

Purification of immunoglobulins

Both chicken and rabbit immunoglobulins were purified by ion exchange chromatography on a DEAE Sepharose column followed by affinity chromatography on cyanogen bromide activated sepharose 4B coupled to rbST.

Purity of anti rbST polyclonal antibodies

The purity of anti rbST polyclonal antibodies was determined by SDS-PAGE analysis (Laemmeli, 1970) and western blot (Towbin *et al*., 1979). The protein content of the sample was determined by Lowry's method (1951). The antibodies were lyophilized and stored at -20°C in aliquots for further use.

RESULTS AND DISCUSSION

In the present study, GH antibody was raised successfully in chickens and rabbits using rbST, Monsanto of NHPP, California, USA. Antibodies against rbST in chicken and rabbit were detected by indirect ELISA. The absorbance obtained by GH antibody positive serum showed more than twice the absorbance obtained by GH antibody negative serum by serial diluting the serum from 1:100 to 1:51200 dilution in duplicate. Purification of antibodies was performed by ion exchange chromatography followed by affinity chromatography. Positive fractions of antibodies were determined by western blot, which revealed a single band of 22 kDa and confirmed the presence of antibodies. The purity of antibodies was checked by SDS-PAGE, which revealed the presence of a single band of 166 kDa for chicken antibody and 135 kDa for rabbit antibody that confirmed the purity of the antibody. Hence, polyclonal antibodies against rbST were obtained for estimation of GH

in serum and milk of buffaloes. Similar polyclonal antibodies have also been used by many workers to determine GH by ELISA (Brambilla *et al*., 1993; Davis *et al*., 1994).

REFERENCES

Brambilla, G.F., A. Berrini, V. Borromeo and C. Secchi. 1993. Detection of growth hormones in the plasma of cows treated with recombinant bovine growth hormone. *Anal. Chem. Acta.*, **275**(1-2): 173-176.

Davis, S.L., N.B. Wehr, D.M. Laird and A.C. Hammond. 1994. Serum growth hormone binding protein (GHBP) in domestic animals as measured by ELISA. *J. Anim. Sci.*, **72**(7): 1719-1727.

Laemmli, U.K. 1970. Cleavage of structure protein during assembly of the head bacteriophage T_4. *Nature*, **227**: 680-685.

Lowry, D.H., N.J. Rsenbrough, A.L. Farr and R.J. Randall. 1951. Protein measurement with the folin phenol reagent. *J. Biol Chem.*, **193**: 265-275.

Masseyeff, R., J. Gombert and J. Josselin. 1965. Methode de preparation de La β-2 macroglobulin serum humain. *Intl. J. Immunochem.*, **2**: 177-180.

Prakash, B.S., M. Mondal and N. Anandlaxmi. 2003. Development and validation of a simple sensitive enzyme immunoassay (EIA) for GH determination in buffalo plasma. *J. Immunoassay Immunochem.*, **23**: 409-420.

Secchi, C., A. Benini, D. Gaggioli, F. Acocella, J. Beattie and V. Barromeo. 1999. Monoclonal antibody capture flurometric enzyme linked immunosorbent assay for detection of porcine growth hormone in plasma. *Anal. Chem. Acta.*, **402**(1-2): 37-45.

Towbin, H., T. Staehelin and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. U.S.A.*, **76**(9): 4350-4354.

Tucker, H.A. 1981. Physiological control of mammary growth, lactogenesis and lactation. *J. Dairy Sci.*, **64**(6): 1403-1421.

Weir, D.M. 1996. *Handbook of Experimental Immunology*, 5th ed. Blackwell Publishing, Boston, USA.

Wilkin, T., C. Casey, A. Tuck. and P. Englefield. 1989. An enzyme-linked immunosorbent assay (ELISA) for measurement of serum antibodies to growth hormone. *J. Immunol. Method.*, **122**(1): 123-127.

EFFECT OF SUPPLEMENTING BYPASS FAT WITH AND WITHOUT RUMEN PROTECTED CHOLINE CHLORIDE ON MILK YIELD AND SERUM LIPID PROFILE IN JAFFARABADI BUFFALOES

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ABSTRACT

Jaffarabadi buffaloes (*n*=27) yielding 8-10 kg milk/head/day were divided into three groups of nine each, based on milk yield, fat per cent and stage of lactation. All animals were fed similar basal diet, comprising 12-15 kg green jowar and 4-6 kg groundnut straw. Concentrate mixture was given according to the level of milk production to meet the maintenance and milk production requirements. Buffaloes in Group 2 were supplemented daily with 150 g bypass fat per animal and in Group 3 along with 150 g bypass fat, 15 g rumen protected choline chloride was also fed. Observations on daily feed intake, daily milk yield, fat per cent etc. were recorded for three months. Average increase in milk yield and fat of Groups 2 and 3 were 1.26 kg ($p<0.05$) and 0.31% ($p<0.05$) and 1.55 kg ($p<0.01$) and 0.44% ($p<0.05$), as compared to Group 1. There was significant improvement in polyunsaturated fatty acids in the milk of Groups 2 and 3. Total unsaturated fatty acids also increased by 9.24 and 9.95% in Groups 2 and 3, respectively. Non-esterified fatty acids in the blood serum decreased by 7.69 and 18.46% ($p<0.05$) in Groups 2 and 3, respectively. There was significant ($p<0.05$) reduction in the cholesterol levels in the blood serum in the animals of Groups 2 and 3, as compared to Group 1. The study indicates supplementing bypass fat helps improving milk and fat yield, which can be further enhanced by fortification with rumen protected choline chloride.

Keywords: bypass fat, rumen protected choline chloride, milk yield, serum lipid profile, Jaffarabadi buffaloes

INTRODUCTION

The beginning of lactation is one of the most crucial periods in the lactation cycle of dairy animals. Despite having access to high energy diets *ad-libitum*, most dairy animals go through a period of negative energy balance, particularly during the first trimester of lactation. The process allows much higher production by changing energy flow so as to partition more energy to milk and less to body reserves for a longer period during lactation. Therefore, nutritional management during this period is crucial for the productivity of dairy animals. Dairy animals mobilize large amounts of fatty acids, also known as non-esterified fatty acids (NEFA), from adipose tissue to meet their energy requirement during early lactation, resulting in increased circulating concentrations of NEFA in the bloodstream.

Supplementation with calcium salts of long chain fatty acids is a good method for increasing energy density of the diet to improve productive

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performances. Choline, a component of phospholipid and methyl donor, plays an essential role in very low density lipoprotein synthesis and thereby contributes to fat export from the liver. Evidence suggests that the dietary supply of choline in early lactating dairy animals may be inadequate, even though choline can be synthesized by the animals (Pires and Grummer, 2008). As dietary choline get degraded in the rumen, it must be supplemented in a protected form (Elek *et al*., 2008). Jaffarabadi buffaloes yield 12-15 litres milk with milk fat in the range of 8 to 12 percent on diets that are usually low in energy density. In view of this, the present study was undertaken in Jaffarabadi buffaloes, to see the impact of supplementing bypass fat with and without rumen protected choline chloride (RPC) on milk yield and blood serum lipid profile.

MATERIALS AND METHODS

Site of study and breed

This study was conducted during August to November months at Galiawada village of Junagadh district in Gujarat State of India. It is sited in western part of India and geographically located between 69.40º to 71.05º east longitude and 20.44º to 21.40º north latitude, at an elevation of 107 metres above mean sea level. The winter temperature varies from 8 to 32ºC while maximum temperatures of 46ºC in summer have been recorded. The average rainfall is 787 mm during the monsoon season. Jaffarabadi *(Bubalus bubalis)* is the heaviest buffalo breed in India. This buffalo breed is native to the region around the Gir forest of Junagadh district. The average daily milk yield of the breed is 12-15 litres, having 8 to 12% fat in milk. Age at first calving, inter calving period and dry period vary from 50-55, 16-18 and 5-8 months, respectively.

Trial design and treatments

A farm-level feeding trial was undertaken to evaluate the effect of supplementing bypass fat, with or without RPC, on milk yield, composition, and blood serum lipid profile in early lactating Jaffarabadi buffaloes. Buffaloes (*n*=27), yielding 8-10 kg milk/head/day were divided into three similar Groups (1, 2 and 3) of nine each, based on level of milk production (8.78 kg), fat (8.17%) and stage of lactation (2-3 weeks post partum). Buffaloes in all the three groups were fed a similar basal diet, comprising 12-15 kg green jowar fodder and 4-6 kg groundnut straw. Concentrate mixture was given according to the level of milk production to meet the maintenance and milk production requirements (Kearl, 1982). No supplement was offered to the buffaloes in Group 1. However, the buffaloes in Groups 2 were supplemented with bypass fat 150 g per animal per day and for those in Groups 3, along with 150 g bypass fat, 15 g RPC was also fed to each of the animals. Feeds and feed supplements were offered to all the animals in three different groups for three months, and observations on daily feed intake, daily milk yield, fat per cent etc. were recorded.

Sample collection and analytical methods

The chemical composition of feeds and fodder offered during the trial period was carried out as per AOAC (2005). Feeds and fodder were also analyzed for neutral detergent fibre (NDF), neutral detergent insoluble nitrogen (NDIN), acid detergent fibre (ADF), acid detergent insoluble nitrogen (ADIN), cellulose, hemi-cellulose and acid detergent lignin (ADL) as per Goering and Van Soest (1970). The degree of rumen protection in bypass fat and RPC supplements were measured as

per procedures of Gulati *et al*. (1993) and Sharma and Erdman (1989). The milk yield of individual buffaloes was recorded in the morning and evening. Milk samples from each buffalo, pooled from each milking were collected weekly for analysis of fat and protein contents by using Milkoscan. Blood samples were collected by jugular venipuncture of each buffalo. The blood was allowed to clot and centrifuged at 1500 rpm for 10 minutes. The serum was then harvested for estimation of serum NEFA, triglyceride and cholesterol using commercially available test kits (Brishketu and Thakur, 2007).

Preparation of fatty acid methyl esters and estimation by using GC

Milk samples were also drawn for preparation of fatty acid methyl esters (FAME), during the trial period. Milk fat from a 2 ml sample was saponified with ethanol and 5N sodium hydroxide, followed by acidification with 5N hydrochloric acid. The collected supernatant was then methylated using methanol. Methyl esters were extracted by adding 3 ml petroleum ether to the solution and removing a portion of the top phase into gas chromatograph (GC) vials. Individual fatty acids were determined by using a Perkin-Elmer gas chromatograph with a flame ionization detector and fitted with a BPX70 capillary column (50 m x 0.32 mm ID). Helium gas was used as a carrier, and the detector temperature was set at 210^{o}C (Ashes *et al*., 1992).

RESULTS AND DISCUSSION

Quality analysis

Analysis of feeds and fodder offered to animals in various groups is given in Table 1. The total fat content in bypass fat supplement was 84.24%, and the degree of rumen protection was 78.76%. The predominant fatty acid in the bypass fat supplement was palmitic acid (43.37%), whereas, levels of oleic, linoleic and linolenic acids were 38.22, 9.64 and 0.22%, respectively. The degree of rumen protection of the choline chloride supplement was 68.29%. Since animals in all the three groups were fed a similar ration, there was no significant difference in daily dry matter intake (DMI) amongst the groups (12.89, 12.36 and 12.72 kg). Other workers too have reported that daily dry matter intake of the ration is not affected by supplementing with rumen bypass fat (Tyagi *et al*., 2009).

Milk production and composition

On supplementing with bypass fat alone or with RPC in lactating Jaffarabadi buffaloes, the average increases in milk yield (kg) were 1.26 ($p<0.05$) and 1.55 ($p<0.01$) in Groups 2 and 3, respectively, as compared to the control. The average fat percent increased by 0.31 ($p<0.05$) and 0.44 ($p<0.05$) in Groups 2 and 3, respectively (Figure 1). As choline is used for phospholipid synthesis, supplementation facilitates lipid absorption and transport, thereby favoring milk fat synthesis. However, the milk protein content remained unaffected amongst the groups. Significant effects of supplementing with bypass fat on milk production and daily fat yield in lactating animals have been reported earlier (Barley and Baghel, 2009; Sirohi *et al*., 2010). Elek *et al*. (2008) and Lima *et al*. (2007) observed significant improvement in milk yield on supplementing with RPC in dairy cows. It is also reported that RPC may improve the milk yield of dairy animals by elevating the export of triglycerides from the liver and by sparing methionine as a methyl donor. Collectively, the study indicated that further improvements in milk

Table 1. Chemical composition (% on DM basis) of feeds and fodder.

Parameter	Cotton seed oil cake	Maize bhardo	Green jowar fodder	Groundnut straw
Moisture	6.46	8.90	82.14	6.62
Crude protein	24.40	8.08	9.59	6.14
Ether extract	6.60	2.88	1.23	0.78
Acid detergent fibre	36.86	2.72	45.48	26.55
Neutral detergent fibre	44.89	17.56	49.51	33.14
Acid detergent lignin	10.64	0.30	14.66	5.23
Cellulose	25.88	2.21	27.61	16.87
Hemi-cellulose	8.03	14.84	4.03	6.59
Silica	0.34	0.21	3.21	4.45
Acid detergent insoluble nitrogen	1.56	0.87	1.92	1.46
Neutral detergent insoluble nitrogen	2.88	1.65	3.55	2.37

Table 2. Milk fatty acid profile (% of total fatty acids).

Fatty acids	Group I	Group II	Group III
Caprylic acid ($C_{8:0}$)	2.36 ± 0.02	2.30 ± 0.03	2.27 ± 0.02
Capric acid ($C_{10:0}$)	3.45 ± 0.03	2.62 ± 0.03	2.69 ± 0.02
Lauric acid ($C_{12:0}$)	2.23 ± 0.01	1.45 ± 0.02	1.29 ± 0.01
Myristic acid ($C_{14:0}$)	12.84 ± 1.20	11.88 ± 1.22	11.95 ± 1.00
Myristoleic acid ($C_{14:1}$)	1.23 ± 0.02	1.25 ± 0.01	1.28 ± 0.02
Palmitic acid ($C_{16:0}$)	30.11 ± 2.22	31.37 ± 3.14	30.40 ± 2.10
Palmitoleic acid ($C_{16:1}$)	1.38 ± 0.03	1.10 ±0.05	0.98 ± 0.03
Stearic acid ($C_{18:0}$)	11.12 ± 0.82	11.08 ± 0.90	10.98 ± 0.85
Oleic acid ($C_{18:1}$)	27.32 ± 1.65	29.68 ± 1.92	29.97 ± 2.12
Linoleic acid ($C_{18:2}$)	1.81 ± 0.04	2.74 ± 0.05	2.73 ± 0.04
Linolenic acid ($C_{18:3}$)	0.71 ± 0.05	0.68 ± 0.05	0.72 ± 0.03
Arachidic acid ($C_{20:0}$)	0.40 ± 0.01	0.39 ± 0.00	0.39 ± 0.00
Total saturated fatty acids	62.51 ± 2.45	61.09 ± 2.44	59.97 ± 3.15
Total unsaturated fatty acids	32.45 ± 3.10	35.45 ± 2.42	35.68 ± 2.31
LCFA ($C_{16:0 \text{ to } 20:0}$)	72.85 ± 2.50	77.04 ± 2.53	76.17 ± 3.14
MUFA ($C_{14:1,\ 16:1,\ 18:1}$)	29.93 ± 2.11	32.03 ± 2.10	32.23 ± 2.55
PUFA ($C_{18:2,\ 18:3}$)	2.52 ± 0.08	3.42* ± 0.06	3.45* ± 0.05

*($p<0.05$)

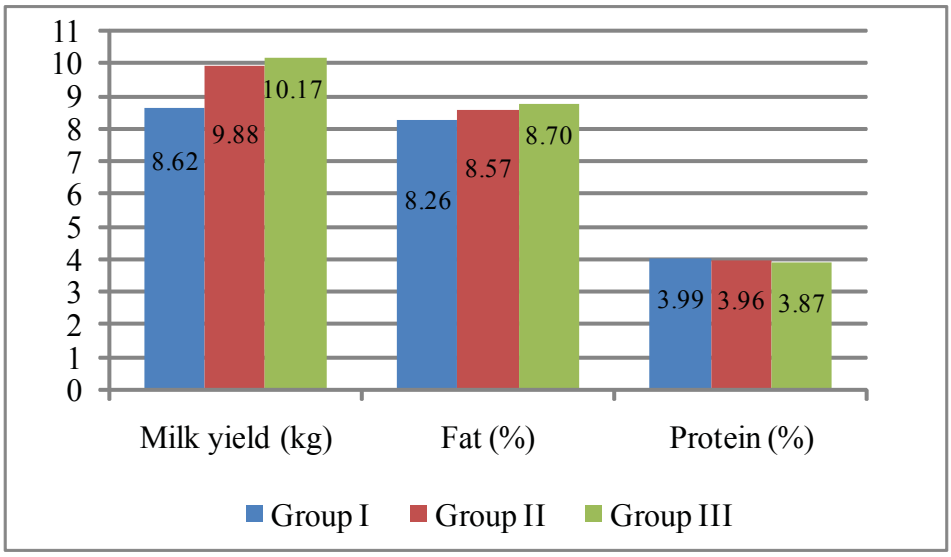

Figure 1. Effect of treatments on milk production and composition.

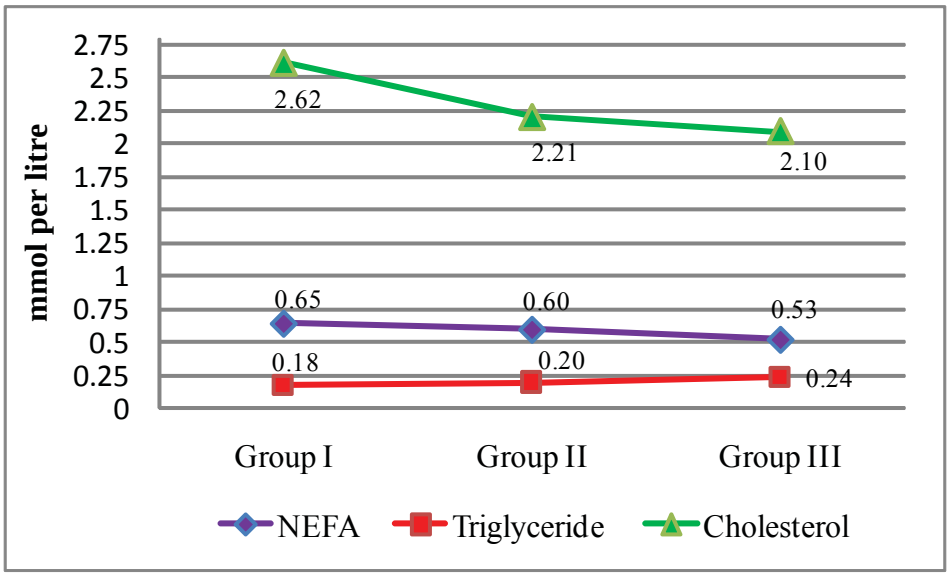

Figure 2. Effect of supplements on blood serum lipids.

production in response to RPC supplementation may be attributed to a methyl donor sparing effect. Thus, enhanced intestinal supply of choline might have further improved milk production in these Jaffarabadi buffaloes.

Milk fatty acid profile

Calcium salts of fatty acids provide partial resistance to lipolysis and bio-hydrogenation in the rumen by ruminal microbes and modify fatty acid profile of milk fat. Significant improvement was observed in poly unsaturated fatty acid (PUFA) content in Groups 2 (35.71%) and 3 (36.90%). Total unsaturated fatty acids increased by about 9.24 and 9.95% in Groups 2 and 3, respectively. Long chain fatty acids (LCFA; $C_{16:0}$ to $C_{20:0}$) and mono unsaturated fatty acids (MUFA; $C_{14:1}$, $C_{16:1}$ and $C_{18:1}$) contents were higher in experimental groups as compared to the control (Table 2). There are several reports indicating that supplementation with bypass fat in the ration of cows and buffaloes increased the proportion of unsaturated and long chain fatty acids of milk fat (Garg *et al*., 2008; Mahecha *et al*., 2008; Sajith *et al*., 2008).

NEFA - An indicator of energy balance and fat mobilization

The presence of NEFA in the blood is a direct indicator of energy balance and massive fat mobilization, suggesting more energy demands than supplied in the diet. Changes in blood serum lipid profile in buffaloes, subjected to the three feeding regimes are presented in Figure 2. Mean serum NEFA level (mmol/l) was reduced to 0.60 and 0.53 in Groups 2 and 3, respectively, as compared to Group 1 (0.65). Significant reduction in serum NEFA level has been reported on feeding RPC (Zahra *et al*., 2006). At the beginning of the lactation cycle, the blood NEFA originating from mobilization of adipose tissue is elevated, mainly due to a negative energy balance. Increased concentrations indicate lipolysis, which occurs in response to increased energy demand. Levels of blood serum triglycerides (mmol/l) increased on feeding bypass fat with and without RPC and cholesterol levels (mmol/l) reduced significantly ($p<0.05$). Brishketu Kumar and Thakur (2007) also reported increased levels of triglycerides on feeding bypass fat supplement to buffalo calves. Cholesterol is a component of the serum lipoproteins and its concentration in serum gives an indication of overall lipoprotein concentrations. Zahra *et al*. (2006) observed reduction in the level of cholesterol on supplementation of RPC to dairy cows. The results obtained in this study are also in agreement with the previous findings of Janovick Guretzky *et al*., 2006 and Pinotti *et al*., 2004.

CONCLUSION

The study revealed that supplementing with bypass fat in the ration of Jaffarabadi buffaloes helped in improving milk yield and fat per cent, which can be further enhanced by supplementing the ration with rumen protected choline chloride.

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REFERENCES

A.O.A.C. 2005. *Official Methods of Analysis*, 18th ed. Association of Official Analytical Chemists, Washington, DC.

Ashes, J.R., P. St-Vincent Welch, S.K. Gulati, T.W. Scott, G.H. Brown and S. Blakely. 1992. Manipulation of the fatty acid composition of milk by feeding protected canola seeds. *J. Dairy Sci.*, **75**(4): 1090.

Barley, G.G. and R.P.S. Baghel. 2009. Effect of bypass fat supplementation on milk yield, fat content and serum triglyceride levels of Murrah buffaloes. *Buffalo Bull.*, **28**(4): 173-175.

Brishketu Kumar and S.S. Thakur. 2007. Effect of supplementing bypass fat on the performance of buffalo calves. *Indian J. Anim. Nutr.*, **24**(4): 233-236.

Elek, P., J.R. Newbold, T. Gall, L. Wagner and F. Husventh. 2008. Effects of rumen protected choline supplementation on milk production and choline supply of periparturient dairy cows. *Animal*, **2**(11): 1595-1601.

Garg, M.R., P.L. Sherasia, B.M. Bhander, S.K. Gulati and T.W. Scott. 2008. Effect of feeding bypass fat supplement on milk production and characteristic of butter fat. *Indian J. Dairy Sci.*, **61**(1): 13-23.

Goering, H.K. and P.J. Van Soest. 1970. *Forage Fibre Analysis (Apparatus, Reagents, Procedures and Some Applications)*, *Agriculture Handbook No. 379*. U.S. Agricultural Research Service, Superintendented of documents, U. S. Government Printing Office, Washington, D.C. 20402.

Gulati, S.K., J.R. Ashes and T.W. Scott. 1993. Optimizing the nutritional value of oilseed proteins for ruminants. Proceedings of 90th American Oil Chemists Society Conference (A.O.C.S.). *INFORM,* **10**: 41.

Janovick Guretzky, N.A., D.B. Carlson, J.E. Garret and J.K. Drackley. 2006. Lipid metabolite profiles and milk production for Holstein and Jersey cows fed rumen-protected choline during the periparturient period. *J. Dairy Sci.*, **89**: 188-200.

Kearl, L.C. 1982. *Nutrient requirements of ruminants in developing countries. International Feedstuffs Institute*, Utah Agricultural Experiment Station, Utah State University, Logan, Utah, USA.

Lima, F.S., M.F. Sa Filho, L.F. Greco, F. Susca, V.J. Magalhaes, J. Garrett and J.E.P. Santos. 2007. Effects of feeding rumen protected choline (RPC) on lactation and metabolism. *J. Dairy Sci.*, **90** (Suppl. 1): 174.

Mahecha, L., J. Angulo, B. Salazar, M. Ceron, J. Gallo, C.H. Molina, E.J. Molina, J.F. Surarez, J.J. Lopera and M. Olivera. 2008. Supplementation with bypass fat in silvopastoral systems diminishes the ratio of milk saturated/ unsaturated fatty acids. *Trop. Anim. Health Pro.*, **40**: 209-216.

Pinotti, L., A. Campagnoli, L. Sangalli, R. Rebucci, V. Dell'Orto and A. Baldi. 2004. Metabolism in periparturient dairy cows fed rumen protected choline. *J. Anim. Sci.*, **13**(l): 551-554.

Pires, J.A.A. and R.R. Grummer. 2008. Micronutrients and their impact on high performing dairy cows - A focus on niacin and choline. *In Proceedings of Southwest Nutrition and Management Conference*, Department of Animal Sciences, University of Arizona.

Sajith, P., A. Kumar and D.P. Tiwari. 2008. Effect of feeding bypass fat (calcium salts of palm

oil fatty acids) on performance of lactating crossbred cows. *Asian Austral. J. Anim.*, **21**: 376-385.

Sharma, B.K. and R.A. Erdman. 1989. *In vitro* degradation of choline from selected feedstuffs and choline supplements. *J. Dairy Sci.*, **72**: 2772-2776.

Sirohi, S.K., T.K. Walli and R.K. Mohanta. 2010. Supplementation effect of bypass fat on production performance of lactating crossbred cows. *Indian J. Anim. Sci.*, **80**(8): 733-736.

Tyagi, N., S.S. Thakur and S.K. Shelke. 2009. Effect of feeding bypass fat supplement on milk yield, its composition and nutrient utilization in crossbred cows. *Indian J. Anim. Nutr.*, **26**(1): 1-8.

Zahra, L.C., T.F. Duffield, K.E. Lesilie, T.R. Overton, D. Putnam and S.J. LeBlanc. 2006. Effects of rumen protected choline and monensin on milk production and metabolism of periparturient dairy cows. *J. Dairy Sci.*, **89**: 4808-4818.

Instructions for Authors

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Sattar, A. 1995. *Studies on the effect of immunopotentiation of vaccinated pregnant buffaloes and cows on neonatal antibody titre and hematological profile.* Ph. D. Thesis, University of Agriculture, Faisalabad, Pakistan. 208p.

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Sabrani, M., K. Diwyanto and M. Winugroho 1994. A critical review of buffalo research and development activities in Indonesia. Past performanceand future strategies, p. 78-89. *In Proceedings of 1st Asian Buffalo Association Congress,* Thailand.

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